Electronic Supplementary Information

A Proof-of-Concept, Two-Tiered Approach for Ricin Detection Using Ambient Mass Spectrometry

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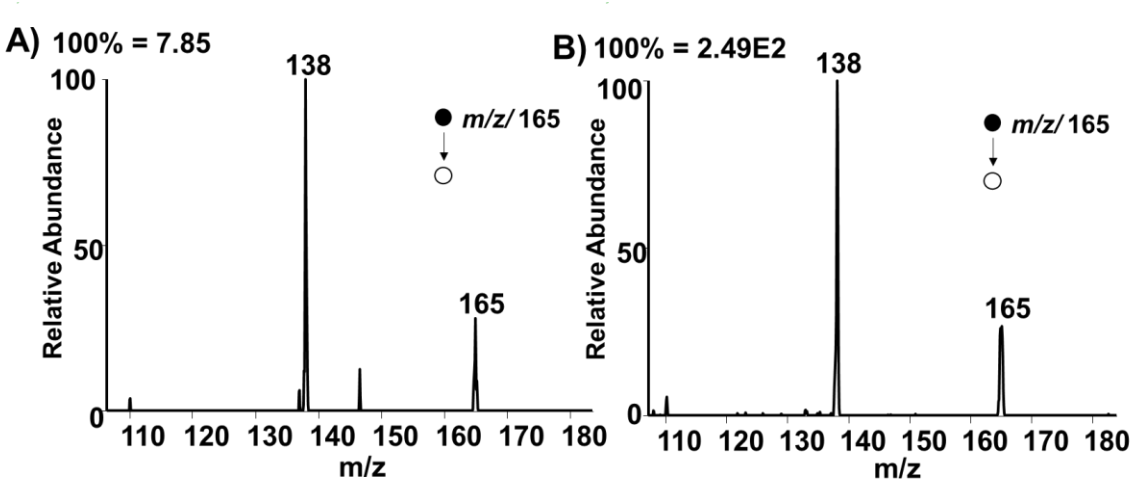
Supporting Information is summarized below:

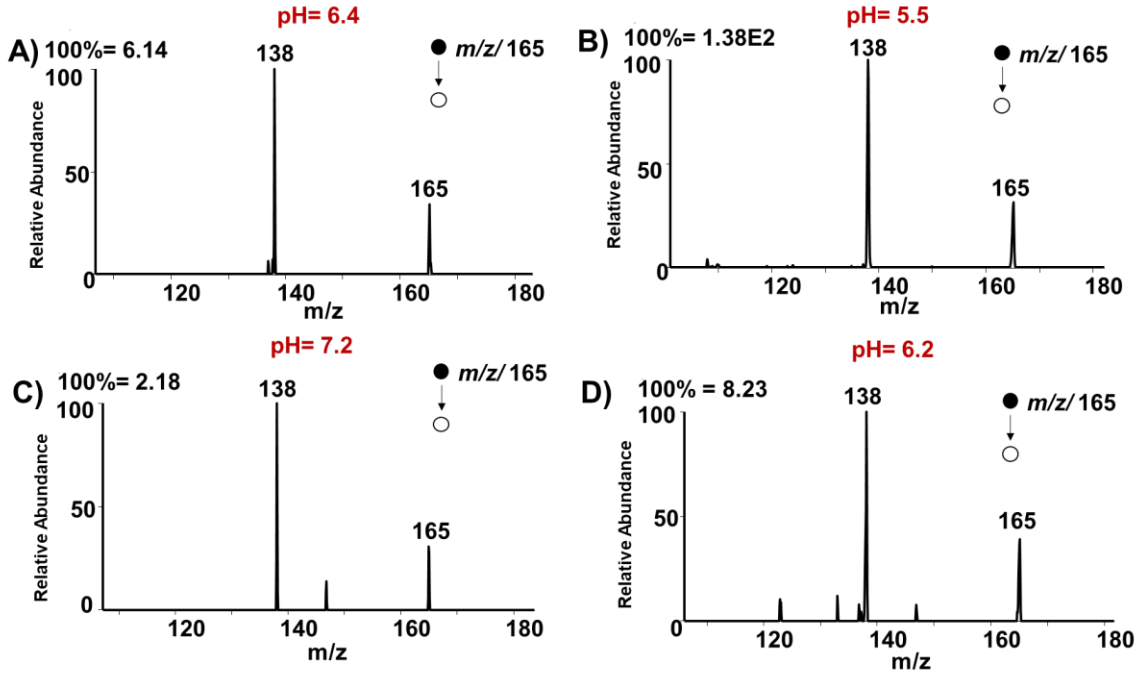
Figure S1. Ricinine ionization efficiency with pure solvents



Tandem mass spectrometry (MS/MS) spectra showing the ionization efficiency of 250 ng/mL ricinine solutions in A) ethyl acetate and B) methanol analyzed with thread spray MS.

For optimizing ricinine detection on the hydrophobic thread, solvent composition was first investigated for pure standard solutions. Given the structure of the analyte (Scheme 1 of manuscript) protonation of the tertiary amine is the most probable during the electrospray process due to its basicity, meaning the solvent needed for analysis must be protic. For the application of biofluid analysis, however, the appropriate solvent must be immiscible in water to selectively extract only the organic analyte molecules, without transferring the aqueous species from the matrix. With these characteristics in mind, the solvent system needed not only has to donate protons but be compatible with biofluid analysis. Initial screening of solvents included ethyl acetate (Supplemental Figure 1A) and methanol (Supplemental Figure 1B) to probe the ionization efficiency of the system – ethyl acetate because of its immiscibility with water and compatibility with biological fluid analysis and methanol because of its protic nature to facilitate protonation. As expected, the analyte had a lower ionization efficiency in ethyl acetate than in methanol due to it being the less protic solvent system.

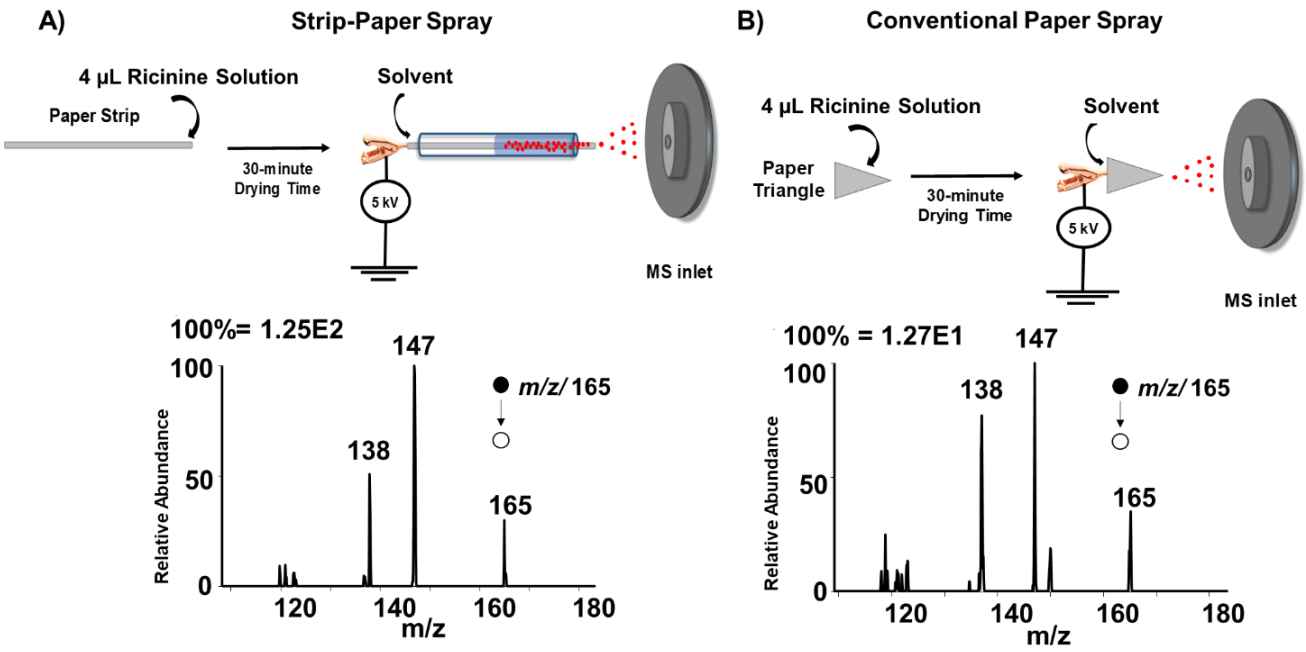
Figure S2. Optimization of acid additives on hydrophobic thread



Tandem MS spectra recorded of 250 ng/mL ricinine solutions using ethyl acetate containing A) acetic acid at pH=6.4, B) acetic acid at pH=5.5, C) formic acid at pH=7.2, and D) formic acid at pH=6.2. Analyses were conducted with hydrophobic thread spray.

To increase the efficiency of the ethyl acetate system, acidic additives were explored, in order to influence protonation. Typically for mass spectrometric coupled liquid chromatography experiments, small volumes of acids, such as formic (pKa= 3.75) and acetic acid (pKa= 4.75), are used to aid in ionization. Given this precedent, and their compatibility with the electrospray process, these acids were added to ethyl acetate for detection. Tandem MS experiments show that on the hydrophobic thread, the incorporation of acetic acid at pH=5.5 (Supplemental Figure 2B) aided in a drastic increase in analyte signal, in comparison to ethyl acetate with formic acid (Supplemental Figures 2C and D). Though the pKa values for these acids differ by 1 full pH unit, we believe the increase in ionization efficiency for the “weaker” acid is due to better wettability of the solvent on the hydrophobic thread. This facilitates a better extraction of the analyte from the thread substrate, increasing the analyte-to-solvent ratio, leading to a higher ion yield during MS analysis. The solvent system with ethyl acetate and acetic acid at pH=5.5 was used for all other studies.

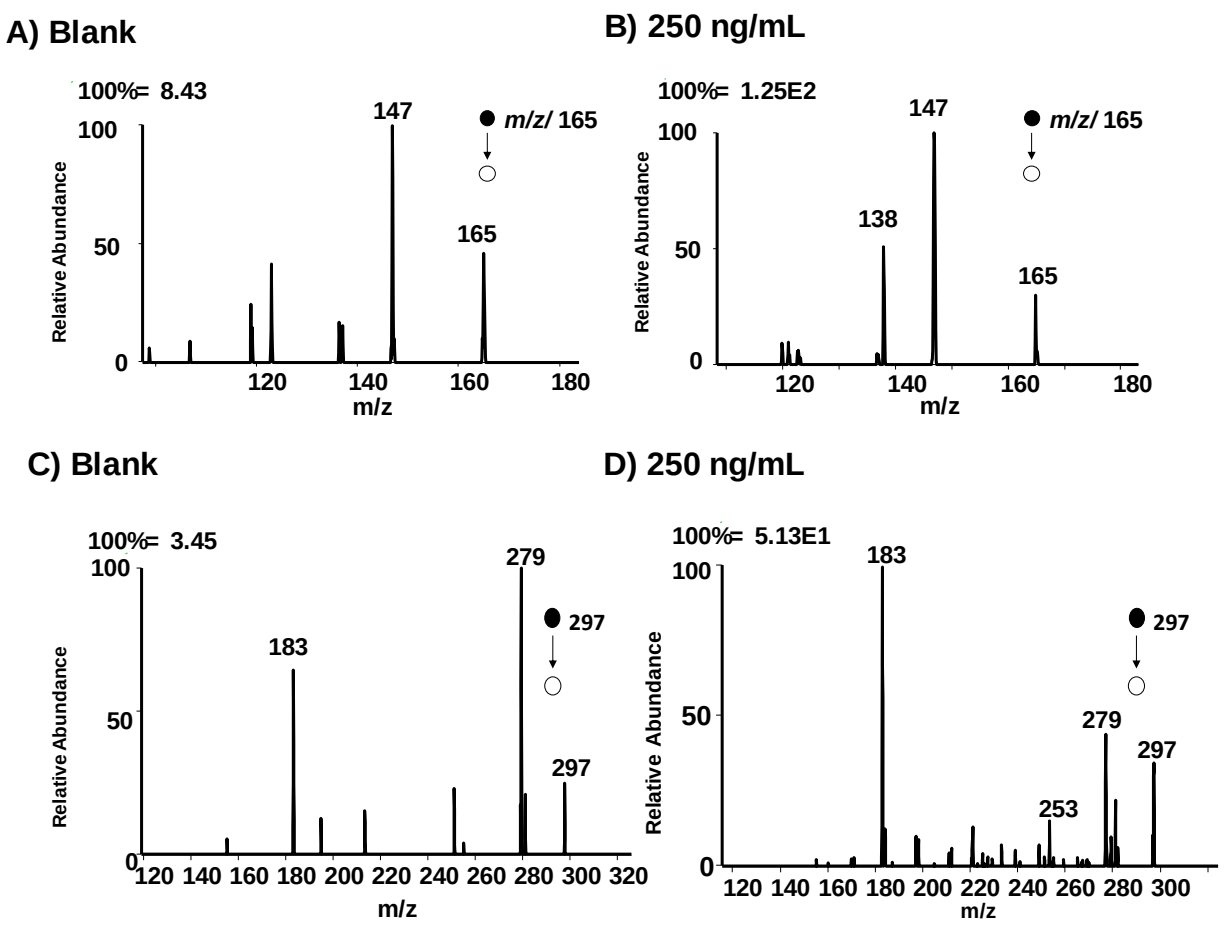
Figure S3. Paper Configuration Comparison



Comparison of paper configurations for ricinine analysis. A) Novel strip-paper spray setup and B) traditional paper spray (PS) were both used to analyze a 250 ng/ mL solution of ricinine with ethyl acetate (pH 5.5) as the spray solvent. Note that the peak at *m/z* 147 is an unrelated background ion.

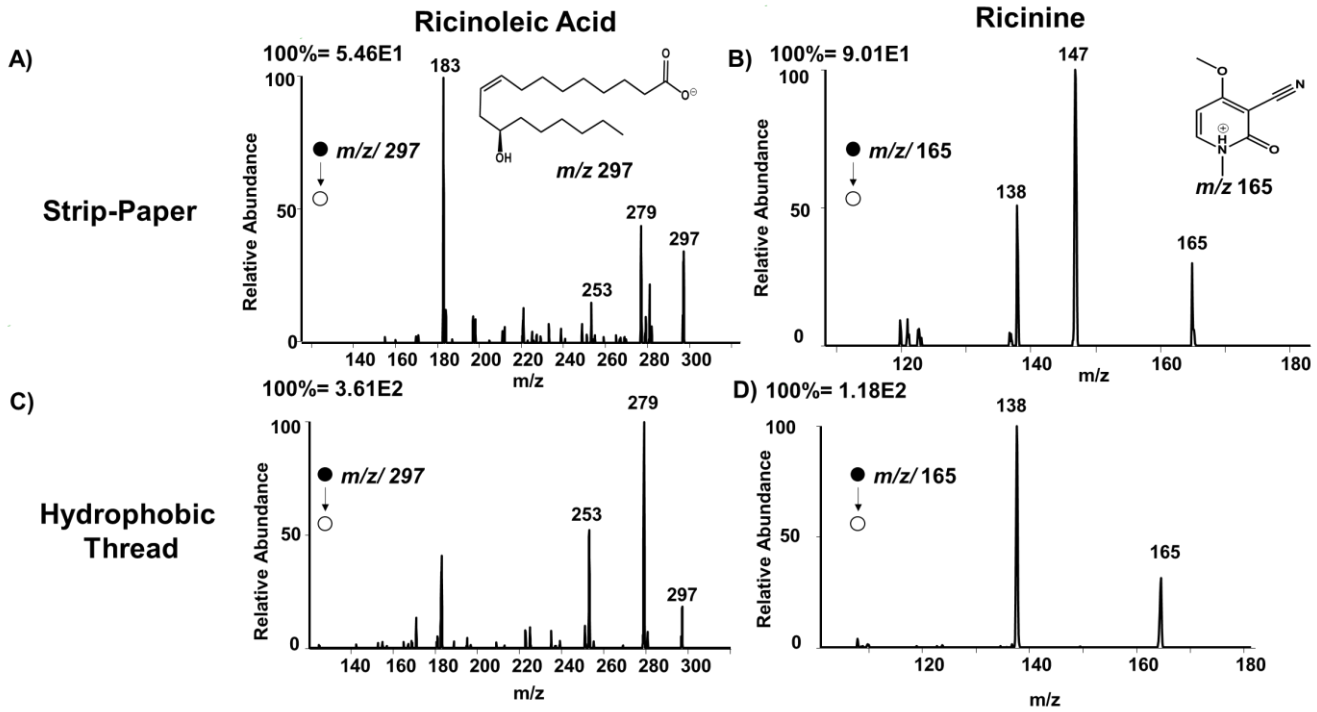
As shown in the figure above, the use of paper strips yields better ionization efficiency than traditional PS. We are attributing this increase in signal with the fact that the substrate is housed inside a glass capillary, allowing for a more efficient liquid-liquid extraction, slower solvent evaporation rate, and a smaller surface area minimizing analyte diffusion processes. While both configurations are viable options, paper strips are advantageous because there is no longer a need to cut the substrate into a triangle or to preserve a sharp, angled tip during sampling, storage, or analysis.

Figure S4. Strip-Paper Spray Solvent Analysis



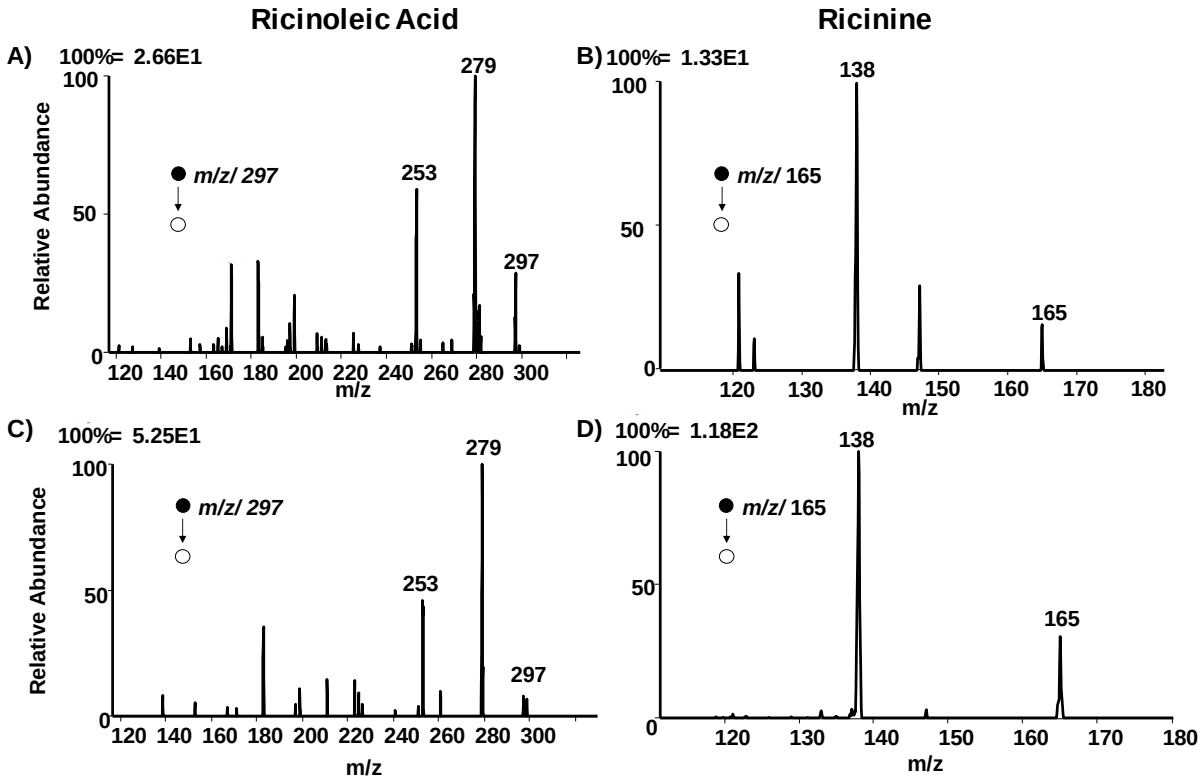
Tandem MS spectra for A) solvent blank and B) 250 ng/mL standard solution of ricinine and C) solvent blank and D) 250 ng/mL standard solution of ricinoleic acid analyzed by paper strips using acidified ethyl acetate (pH= 5.5). Blanks were run in order to characterize any isobaric ions and fragments that could potentially interfere with ricinine analysis/quantification. On paper substrates, a strong signal for the fragment ions (*m/z* 147 for ricinine and *m/z* 183 for ricinoleic acid) were seen in all MS/MS spectra during this study and are unrelated to the analytes.

Figure S5. Strip-Paper and Thread Spray Analysis



Comparison of ambient methods for pure standard solution analyses of ricin biomarkers (250 ng/mL concentrations). A) and B) show MS/MS spectra of ricinoleic acid and ricinine using strip-paper spray and C) and D) are spectra for both biomarkers using hydrophobic thread spray MS. As shown, both methods are viable for analysis using our two-tiered approach, even though the paper substrate yields a lower ion yield and is subject to minimal matrix effects.

Figure S6. Mixture Analysis



Two-tiered approach applied to the analysis of 1:1 ricinoleic acid and ricinine (250 ng/mL) mixtures. A) and B) were analyzed in a pure solution using paper strips and C) and D) were analyzed in serum using hydrophobic thread.

For these experiments, ricinoleic acid was analyzed first in negative mode followed by positive mode analysis of ricinine. It is important to note that the spray solvent contains acetic acid which plays a role in the lower intensity, noisier negative mode spectra. Even with the incorporation of acid, however, the appropriate fragmentation ions for ricinoleic acid are present during screening tests.